Clinical and histologic evaluation of different approaches to gain keratinized tissue prior to implant placement in fully edentulous patients

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Abstract: OBJECTIVES: This work aimed to investigate the effectiveness and predictability of different treatment modalities for gain of keratinized tissue (KT) in fully edentulous jaws prior to dental implant placement: apically positioned flap (APF), APF plus xenogeneic collagen matrix (XCM), and APF plus free gingival graft (FGG). MATERIALS AND METHODS: In fully edentulous patients with insufficient zones of KT at the prospective implant positions, four treatment modalities were performed in the lower jaw: APF, XCM, FGG, and an untreated control group (control). APF and XCM were applied in the first molar positions, FGG and control in the canine positions. Assessed outcomes up to 3 months post-surgery included changes in width of KT (over a 3-month period), histomorphometric analysis of harvested soft-tissue biopsies (at 3 months postoperatively), and patient-reported outcomes measures (PROMs). RESULTS: For the primary outcome, changes in KT width demonstrated an increase of 1.93 ± 1.6 mm (APF), whereas XCM and FGG showed an increase of 4.63 ± 1.25 mm and 3.64 ± 2.01, respectively. Histomorphometric analyses revealed a thickness of the epithelium ranging between 375 ± 122 μm (APF), 410 ± 116 μm (XCM), 336 ± 122 μm (FGG), and 413 ± 109 μm (control). All biopsies showed a regular muco-periosteal structure with a keratinized epithelium of comparable thickness in all groups. CONCLUSION: All three methods were suitable to increase the width of KT, although APF alone rendered roughly 50% less gain compared to XCM and FGG. CLINICAL RELEVANCE: The use of XCM in conjunction with an APF represents a valuable treatment option for the gain of keratinized tissue in edentulous sites.

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Clinical and histologic evaluation of different approaches to gain keratinized tissue prior to implant placement in fully edentulous patients

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Running title: increase of keratinized tissue

Key words: collagen, matrix, augmentation, keratinized tissue, keratinized mucosa, autologous, gingival graft, transplantation, Patient-reported outcome measures

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ABSTRACT

Objectives: to investigate the effectiveness and predictability of different treatment modalities for gain of keratinized tissue (KT) in fully edentulous jaws prior to dental implant placement: apically positioned flap (APF), APF plus xenogeneic collagen matrix (XCM), APF plus free gingival graft (FGG).

Materials & Methods: In fully edentulous patients with insufficient zones of KT at the prospective implant positions, four treatment modalities were performed in the lower jaw: APF, XCM, FGG and an untreated control group (control). APF and XCM were applied in the first molar positions, FGG and control in the canine positions. Assessed outcomes up to 3 months post-surgery included: changes in width of KT (over a 3-month period), histomorphometric analysis of harvested soft tissues biopsies (at 3 months postoperatively) and patient-reported outcomes measures (PROMs).

Results: For the primary outcome, changes in KT width demonstrated an increase of 1.93 mm ±1.6 mm (APF), whereas XCM and FGG showed an increase of 4.63 mm ± 1.25 mm and 3.64 ± 2.01, respectively. Histomorphometric analyses revealed a thickness of the epithelium ranging between 375±122 um (APF), 410±116um (XCM), 336±122 um (FGG) and 413±109um (Control). All biopsies showed a regular muco-periosteal structure with a keratinized epithelium of comparable thickness in all groups.

Conclusion: All three methods were suitable to increase the width of KT, although APF alone rendered roughly 50% less gain compared to XCM and FGG.

Clinical Relevance: The use of XCM in conjunction with an APF represents a valuable treatment option for the gain of keratinized tissue in edentulous sites.

INTRODUCTION

Controversial results are reported with respect to the question whether or not there is a need for keratinized tissue around dental implants [1, 2]. Various studies suggested associations between an adequate width of keratinized tissue, higher survival rates of dental implants, the health of the peri-implant tissue, and an improved esthetic outcome [3-5]. More recent studies demonstrated that implants with a reduced width of peri-implant keratinized tissue were more prone to plaque accumulation and bleeding as well as soft tissue recessions [6-9]. In a randomized clinical study, free gingival grafts (FGGs) significantly increased the width of keratinized mucosa around dental implants. Moreover, implant sites augmented with FGGs showed a significantly reduced mucosal inflammation and less alveolar bone loss [10]. In order to facilitate patients' oral hygiene and to maintain the keratinized mucosa level, techniques to increase the peri-implant keratinized tissue have to be considered. Traditionally, grafting procedures are performed using autogenous soft tissue transplants [11-13]. However, surgical difficulties, lack of color match, and an increased patient morbidity represent major disadvantages when using autogenous transplants [14-18]. In order to overcome these issues encountered with autogenous grafting, various techniques and biomaterials of allogenic and xenogeneic origin were introduced and evaluated in clinical studies to increase the width of keratinized tissue [19-24]. Collagen devices from xenogeneic origin have been successfully used in dentistry as barrier membranes [25-27], as socket seal [28-30] and to gain keratinized tissue [19, 21-23, 31, 32]. Even though, results from clinical trials demonstrated that a collagen matrix (XCM) in combination with an apically positioned flap (APF) was effective and predictable for attaining a band of keratinized tissue [21, 23, 32, 33], a clinical benefit compared to the APF alone has not been shown so far.

The aim of the present pilot study was, therefore, to evaluate four treatment modalities, an apically positioned flap (APF), APF plus XCM, APF plus FGG and an untreated control

group, in prospective implant positions and to report histologic outcomes three months following the surgical interventions.

MATERIALS AND METHODS

Study design

The present study was designed as a split-mouth pilot case series to evaluate three procedures for gain of keratinized tissue and to histologically describe the tissues three months after the surgical interventions. The local ethics committee approved the study protocol and procedures (KEK-Nr. 2011-0124/5). Patients meeting the following inclusion criteria were consecutively recruited and enrolled at the Clinic of Fixed and Removable Prosthodontics and Dental Material Science, University of Zürich, Switzerland, between 2011-2012:

- Signed informed consent
- The patient (male or female) must be 18 years or older
- The patient is able to comply with the study-related procedures such as exercising good oral hygiene and attending all follow-up procedures
- The patient is able to fully understand the nature of the proposed surgery and is able to provide a signed informed consent.
- Fully edentulous patients in need of implant therapy in the mandible and ability to place dental implants in the two canine and the two first molar positions
- A reduced width of keratinized tissue (<2mm), measured from the center of the four prospective mandibular implant positions (canines and first molars) to the buccal mucogingival junction
- Well-fitted, new maxillary and mandibular prostheses
- Generally healthy
- Commitment to maintain good oral hygiene
- No systemic disease that could affect wound healing and prevent implant placement

Exclusion criteria for all subjects included:

- Patient is a heavy smoker (> 10 cigarettes per day)
- Patient is an insulin dependent diabetic
- General contraindications for dental and/or surgical treatment are present.
- The patient has a history of malignancy, radiotherapy, or chemotherapy for malignancy within the past five.

- The patient is pregnant or nursing
- The patient is taking medications or having treatments, which have an effect on mucosal healing in general (e.g. steroids, large doses of anti-inflammatory drugs).
- The patient has a disease, which affects connective tissue metabolism (e.g. collagenases).
- The patient is allergic to collagen.
- Patients having participated in a clinical trial within the last six months.

Patients not meeting all inclusion criteria were excluded from the study. Upon enrollment, alginate impressions of the mandible were obtained and stents for measurements were digitally designed and fabricated with a 3D printer (Figure 1). The stent was designed in such a way that it allowed measuring the width of keratinized tissue from the center of the prospective implant positions to the buccal mucogingival junction.

Surgical intervention

At the day of surgery, patients rinsed with 0.2% of chlorhexidine solution and were given medication for pain relief (Mefenamic acid, 500mg). Subsequently, photographs of the four sites were taken, and the width of keratinized tissue was measured on the buccal side of the four prospective implant positions using a caliper and the prepared stent. Following local anesthesia, four treatment modalities were then randomly applied to the left and right side of the mandible:

Molar positions:

- Apically positioned flap (APF)
- APF plus collagen matrix (XCM) (Mucograft® Geistlich Pharma AG, Wolhusen, Switzerland)

Canine positions:

- APF plus autogenous free gingival graft (FGG)

- No surgical intervention (control)

In group APF (Figure 2), a split-flap was prepared leaving 1mm of keratinized tissue (KT) at the coronal border of the APF and sutured apically using non-resorbable sutures (Dafilons 5-0, B. Braun Melsungen AG, Melsungen, Germany). The periosteum was exposed with a height of 7mm and a width of 10mm and left for spontaneous healing. At XCM sites (Figure 2), the same procedure (split-flap with 1mm of KT) was performed. Subsequently, the XCM was shaped to a final dimension of 5x10mm and sutured coronally. A gap of 2mm was left for spontaneous healing between the apical border of the XCM and the coronal border of the split-flap. One or two cross-section sutures were placed on top of the XCM to stabilize the matrix on the wound bed. In the FGG group (Figure 2), a similar APF was performed (including 1mm of KT). A FGG with a thickness of 1.5mm and a dimension of 5x10mm was harvested from the palate. The FGG was sutured coronally and stabilized with one or two cross-section sutures. Similar to XCM sites, 2mm of periosteum were left for healing by secondary intention. One site was left as a negative control group (no further intervention) (Figure 2).

Patients were given medications for pain relief (Mefenamic acid, 500mg every 8 hours), as well as a disinfectant solution (Chlorhexidine digluconate, 0.2% solution for rinsing every 8 hours for a period of 7 days). No adaptations were made to the mandibular prostheses and patients were allowed to wear them during the entire study period. At day 7 post-surgery, all patients were recalled for suture removal, clinical measurements and clinical photographs.

Outcome measures

Width of keratinized tissue

The width of keratinized tissue at the four sites was recorded to the nearest 0.1mm at baseline (prior to surgery), after surgery, at 7 days, 30 days, and at 90 days using a caliper and 3D-printed stent (Bio-compatible PolyJet photopolymer (MED610),

Stratasys, MN, USA) to standardize the measurements for reference and accurate reproducibility (Figure 1). The measurements were performed 5 times at a 1mm distance and per site. One examiner not involved in the clinical procedure performed all the measurements. Intra-examiner reproducibility was assured by pre-operative calibration and training program on 3D-printed stent insertion, stabilization and consistent data collection.

Patient-reported outcome measures

Patient-reported outcome measures (PROMs) were evaluated post-surgically. For that purpose, a questionnaire was handed out and explained to the patients. Assessed parameters included bleeding, swelling and pain. Patients recorded their binary answers (yes/no) for bleeding (immediately post-surgery and on the following day) and for swelling (post-surgery to 7 days). Patients' pain experience was evaluated using a visual analogue scale (VAS) ranging from 0-10, with 0 representing the absence of pain, 1-3 for minimal pain, 4-6 for moderate and 7-10 for severe pain.

Histologic processing and analysis

At 90 days following soft tissue augmentation, clinical measurements and photographs were obtained (Figure 2). In the four prospective implant positions, soft tissue biopsies were obtained using a biopsy drill (diameter 1.8mm; depth 5mm). Specimens were placed in 10% neutral-buffered formalin solution for fixation. Subsequently, implant placement was performed according to the manufacturer's recommendation (data not reported here). After fixation, each sample was processed and dehydrated in alcohol solutions of increasing concentration, cleared in isoparaffin H and embedded in paraffin. Embedded samples were cut at 5 µm using a microtome (MICROM®, France), sections were prepared and stained with H&E.

All histological sections were evaluated using a Nikon microscope (ECLIPSE E600, Nikon, Egg, Switzerland) for qualitative and semi-quantitative histological analysis. For histomorphometric analysis, the digitized histological images were analyzed using an image-processing program (Image J, NIH, Bethesda, USA), to assess the thickness of keratinized epithelium (Figure 3).

Statistical analysis

Statistical analyses were performed descriptively to compare the different treatment modalities at 30 and 90 days and for changes over time. Current statistics (mean, standard deviation, median and IQR) were used to describe the quantitative parameters of keratinized tissue width and microscopic thickness of the epithelium (day 90). Due to the small sample size, no further statistical tests were performed.

Results

A total of 9 patients (36 sites) in fully edentulous patients with an age range between 46-88 years (mean 66.3 years) were included in the study and underwent soft tissue augmentation surgeries. All patients received 4 treatment modalities, healing was generally uneventful and no local infection was observed at suture removal. Patient-related outcomes at day 7 postoperatively were collected in form of a questionnaire. In 33% of the sites (irrespective of the treatment modality), moderate to severe pain was reported. Absence of pain was reported in 13.9 % of the sites only (Table 1).

Width of keratinized tissue

All data are presented in Table 2. Immediately post surgery, the gain in KT obtained due to the surgical interventions ranged between 3.69mm ± 1.62mm (APF), 4.31mm ± 0.76mm (XCM), 4.44mm ± 1.44mm (FGG) and 0.0mm ± 0.0mm (control).

Between preoperative and 90 days, the gain in width of KT up to 90 days was 1.93 mm ±1.6 mm for APF, 4.63 mm ± 1.25 mm for XCM, 3.64 ± 2.01 mm for FGG and 0.13mm ± 0.31mm for control.

From postoperative to day 90, the width of KT decreased by 35.5 % for APF, increased by 6.8 % for XCM, and decreased by 16.8% for FGG and increased by 3.4 % for control sites.

Descriptive histology

Eight out of 9 patients agreed to take biopsies at 90 days post surgery. In all 8 patients, 4 biopsies were collected at the prospective implant positions. A total of 24 biopsies could be processed and analyzed. This included 5 APF, 5 XCM sites, 7 FGG sites and 7 control sites. Microscopically, the harvested soft tissues appeared to be healthy. In the most coronal part of the biopsy, the *oral epithelium* had a regular appearance with all four components, a keratinized stratum corneum with a keratin layer, a stratum granulosum, a stratum spinosum and a stratum basale. Rete pegs were present, but had an irregular appearance in most cases. The *subepithelial connective tissue* appeared to have a loose structure with relatively thin bundles of collagen fibers in most biopsies, whereas in some samples, thick bundles of collagen fibers were surrounded by an increased number of blood vessels. Few inflammatory cells (macrophages, lymphocytes, granulocytes) were present in the most coronal portion of the connective tissue compartment. In APF and XCM sites, the structure and shape of the epithelium underlying connective tissue appeared to be quite consistent, while in control and FGG sites, more inconsistencies and irregularities of shape and form were visible. Some remnants of the collagen matrices were observed in XCM sites in the most coronal part of the connective tissue.

Histomorphometric outcomes

The histomorphometric analysis revealed a thickness of the epithelium ranging between 375±122 um (APF), 410±116um (XCM), 336±122 um (FGG) and 413±109um (control) at 90 days post surgery (Figure 4A-D).

Discussion

The present study evaluating three methods to increase the width of keratinized tissue in fully edentulous mandibles revealed that: i) in posterior sites, XCM was more effective in terms of gain of KT than APF alone, ii) in anterior sites, FGG resulted in a gain similar to XCM sites in the posterior region, iii) postoperative bleeding occurred frequently and irrespective of the treatment modality, iv) absence of pain was more frequently observed for APF and XCM sites than for FGG sites, v) similar histologic outcomes in all groups at 90 days post-surgery.

To date, there is no data available assessing the use of collagen matrix in fully edentulous jaws prior to implant placement, aiming at gain of keratinized tissue. Moreover, the clinical and patient benefit of an XCM compared to an apically positioned flap alone is unknown. The APF procedure, without incorporating any additional grafting material (XCM, FGG) covering the wound bed, resulted in the least gain of keratinized tissue. Few studies showed similar outcomes when using an APF to regenerate KT in partially edentulous patients [34, 35]. In a case report of 3 patients, an average of 1.5 mm of KT was gained 3-4 weeks post-APF procedure [35]. Another clinical study showed an increase of 2 mm, after 3 months of APF procedure, that decreased to 1.15 mm at the 12-month follow up [34]. Bearing in mind that a minimum of 2 mm width of KT is generally considered to be sufficient and that it is however unknown if a wider band of KT is clinically more advantageous [8, 9, 36], the obtained gain through an APF might still have its clinical indication.

Collagen matrices were applied for various clinical indications, including gingival recession coverage and to increase the width and thickness of gingival/ peri-implant keratinized mucosa [12, 19-22, 37]. The present XCM has a 3-dimensional structure to support the tissue growth, while allowing the cellular adhesion and infiltration into its interconnected porosity [38]. It is made of a collagenous framework that is biocompatible and biodegradable to be replaced by native tissue [20, 38-40]. The

present clinical findings demonstrated an increase in KT width by more than double compared to APF alone. It might in part be explained by the previously mentioned favorable biologic and chemical properties.

Traditionally, autogenous free gingival grafts were considered to be the gold standard for its high success and predictability, as well as the lack for comparable alternatives [11, 12, 41-43]. In the present study, XCM regenerated a width of keratinized tissue that was comparable to the FGG sites at 3 months. These findings are in line with previous clinical studies [19-21, 23, 32, 43]. Since the sites (FGG at canine positions; XCM at molar positions) were not randomized and differed between the area/treatment modalities and based on the pilot character of the study, the results were only compared descriptively without applying further statistical tests. This pilot study could however be of value for the power calculation and design of future studies.

It is accepted to expect some degree of wound contraction and augmentation shrinkage irrespective of the procedure and materials used. Postsurgical soft tissue changes in width and overall shrinkage are a valuable indicative of technique predictability. Literature has shown a wide variation in the amount of shrinkage associated with similar or different augmentation procedures [12]. The present findings showed shrinkage rates of 35.5% for APF and of 16.8 % for FGG sites, 3 months postoperatively. In contrast, XCM sites revealed an increase of 7% relative to the postoperative width of KT. Variations in soft tissue changes could be influenced by a number of factors: the surgical technique (APF vs. APF/FGG vs. APF/XCM), the augmentation site (anterior vs. posterior, periodontal vs. peri-implant), type (XCM, FGG), the characteristics of the grafting material (thickness and prevascularity) and the amount of bone atrophy in the surgical area that could sustain unfavorable muscle attachment [44]. APF, XCM and FGG were reported to exhibit the majority of shrinkage within the first 3 months post surgery [23, 33, 34, 43]. Published data indicated, for XCM and FFG, a maximal shrinkage of 67% and 60%, respectively, one month postoperatively [23], a shrinkage of 20% at 2 months (XCM) [33] and a shrinkage of 34% (XCM) and 28.6% (FGG) at 3 months [21].

All these shrinkage rates are higher than the ones observed in the present study. One possible explanation includes that all patients were fully edentulous and wearing their prostheses as opposed to the above-mentioned studies including tooth and implant sites in partially edentulous patients. The prostheses in the present study might have served as an artificial wound dressing by keeping a continuous pressure on the wound bed and by stabilization the augmented area. Another important factor is that most of clinical studies, including ours, presented relatively short-term findings. Although long-term studies are limited, a 5-year clinical study showed continuous but minimal rates of dimensional changes after the first 3 months of the treatment. The XCM-treated sites showed more significant reduction in KT compared to FGG at 3-, 4- and 5-year follow up visits. Yet, KT maintained an adequate and supportive peri-implant keratinized tissue in both groups [21].

Histologically, the biopsies showed a similar keratinization of multilayer epithelial cells, as well as a normal maturation of connective tissue structures. These histologic findings have been consistently described in previous studies assessing biopsies taken following augmentation with XCM and FGG [19, 20, 43]. The histomorphometric analysis also revealed XCM-site biopsies to have a slightly thicker epithelial layer (410±116 um) compared to sites with FGG (336±122 um) and APF alone (375±122 um). Moreover, the data were comparable to sites without surgical intervention (control sites: 413±109 um). These findings demonstrate that within a 3-month period, irrespective of the treatment modality, stable soft tissue conditions can be expected. This is in line with observations from previous in vivo studies comparing collagen matrices and sites healing spontaneously that demonstrated no differences at the respective study endpoints between 1 and 6 months [45, 46].

Patients' perception on dental care is becoming a vital tool and central outcome in clinical studies. In the present study, each patient received 3 surgical interventions within one appointment. Despite the augmented effect of different within-patient interventions, bleeding, swelling and pain were within 'well-tolerated' range. In general,

patients had the perception of ''mild-moderate'' within the first week postoperatively. Moreover, patients reported absence of pain more frequently with APF and XCM compared to FGG. Postoperative bleeding and swelling occurred frequently, and irrespective of the treatment modality. These data are further limited and need to be interpreted with caution due to the lack of an overall randomization and a relatively low number of patients. It is also more indicative for future studies to investigate and compare similar anatomical sites for the intervention of interest, over a longer period of follow up.

CONCLUSIONS

All three methods were suitable to increase the width of **KT**, although APF alone rendered roughly 50% less gain compared to XCM and FGG. From a histologic point of view, all three-treatment modalities led to matured and stable soft tissues similar to native gingival tissue three months following the surgical interventions.

Compliance with Ethical Standards

Conflict of Interest: Daniel S. Thoma declares that he has no conflict of interest. AbdulMonem Alshihri declares that he has no conflict of interest. Alain Fontolliet declares that he has no conflict of interest. Christoph H. F. Hämmerle declares that he has no conflict of interest. Ronald E. Jung declares that he has no conflict of interest. Goran I. Benic declares that he has no conflict of interest.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research

committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

FIGURE LEGENDS

Figure 1

3D printed surgical stent for reproducible clinical measurements, inserted on an edentulous mandible. Treatment modalities: lower left: FGG; lower right: control; upper left: APF; upper right: XCM. APF = apically positioned flap; XCM = xenogeneic collagen matrix; FGG = free gingival graft.

Figure 2

Clinical situation at 90 days. Treatment modalities: lower left: FGG; lower right: control; upper left: XCM; upper right: APF. APF = apically positioned flap; XCM = xenogeneic collagen matrix; FGG = free gingival graft.

Figure 3

Histologic section (H&E staining), showing the epithelial measurements (magnification 50 X).

Figure 4

A-D: Histologic sections (H&E staining) 90 days after surgery (original magnification x50). A: apically positioned flap (APF) site. B: XCM (xenogeneic collagen matrix) site. C: FGG (free gingival graft) site. D: control site.

Table 1

7-day postoperative patient-related outcome measures. APF = apically positioned flap; XCM = xenogeneic collagen matrix; FGG = free gingival graft.

Table 2

Width of keratinized tissue (Δ mean mm, standard deviation (SD), median, quartile 1 and quartile 3). APF = apically positioned flap; XCM = xenogeneic collagen matrix; FGG = free gingival graft; SD = standard deviation; Q1 = quartile 1; Q3 = quartile 3.

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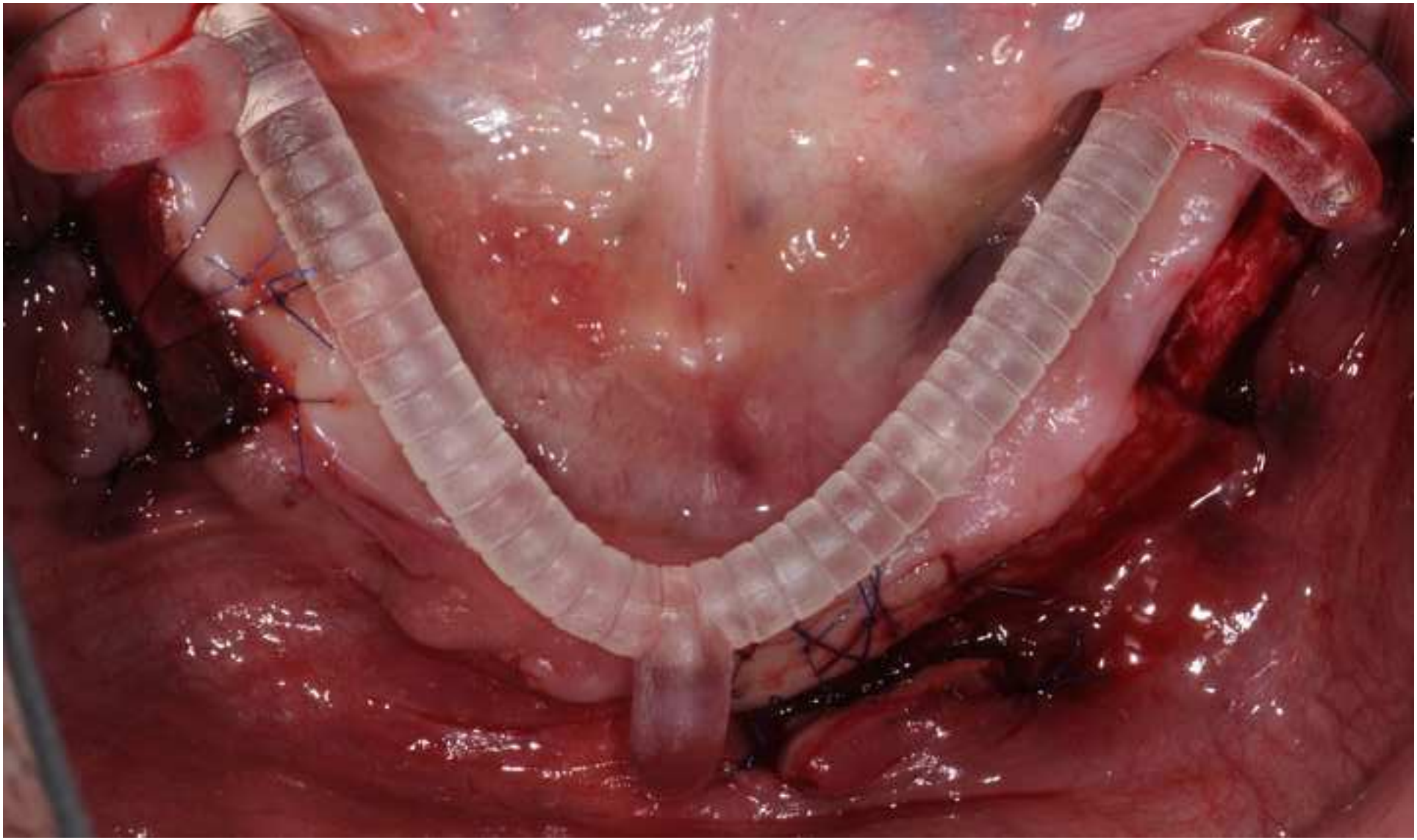

Figure 1

Figure 2

Figure 3

Figure 4A

Figure 4B

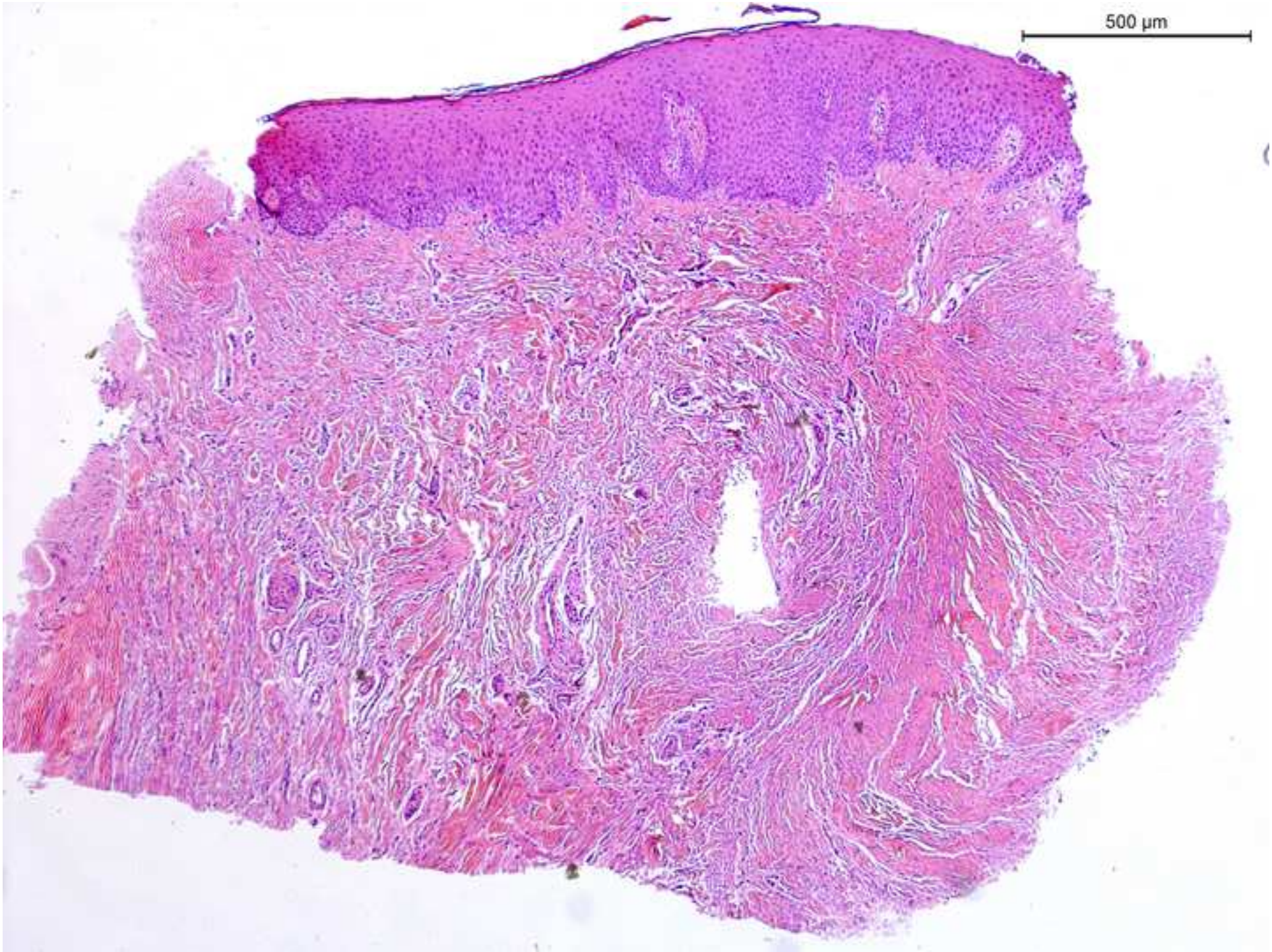

Figure 4C

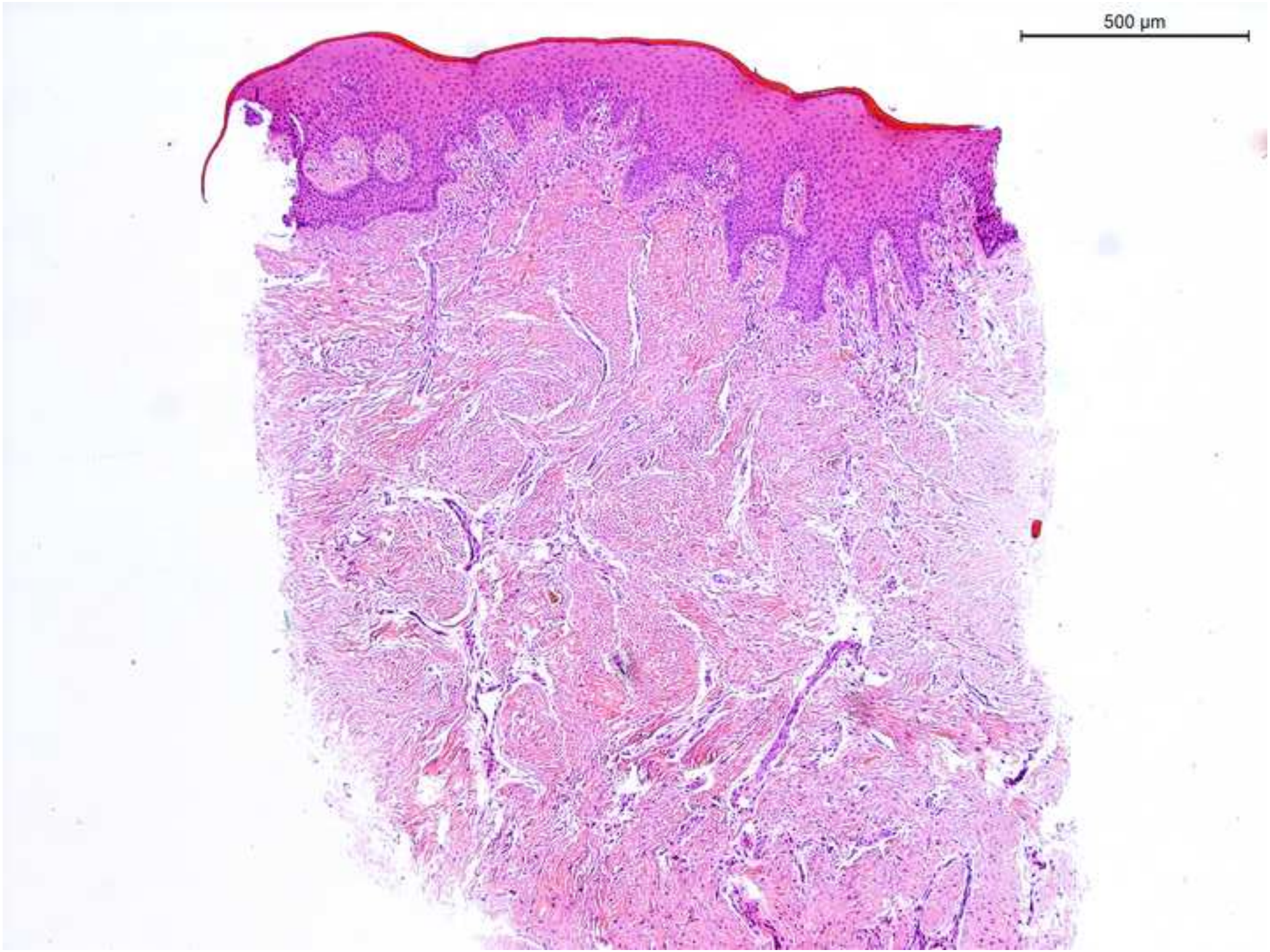

Figure 4D

Table 1

	Bleeding immediately post-surgery	Bleeding 1-day post-surgery	Swelling	Pain Absent 0	Pain Minimal 1-3	Pain Moderate 4-6	Pain Severe 7-10
APF	3 (37%)	1 (12.5%)	3 (37%)	2 (25%)	3 (37%)	3 (37%)	0
XCM	3 (37%)	1 (12.5%)	5 (62.5%)	2 (25%)	3 (37%)	2 (25%)	1 (12.5%)
FGG	4 (50%)	2 (25%)	5 (62.5%)	1 (12.5%)	4 (50%)	3 (37%)	0

Table 2

Treatment modality	Observation period	Change in width of keratinized tissue (mm)				
		Δ Mean	SD	Median	Q1	Q3
APF	Pre- to Postoperative	3.69	1.62	4	3.4	5
XCM	Pre- to Postoperative	4.31	0.76	4.6	3.8	5
FGG	Pre- to Postoperative	4.44	1.44	4.85	4.15	5.2
Control	Pre- to Postoperative	0	0	0	0	0
APF	Preoperative to 30 days	3.1	1.71	2.7	1.75	3.95
XCM	Preoperative to 30 days	4.35	1.81	3.9	3.1	4.75
FGG	Preoperative to 30 days	4.071	1.43	4.8	3.1	5.15
Control	Preoperative to 30 days	0.16	0.29	0	0	0
APF	Preoperative to 90 days	1.93	1.6	1.7	0.7	2.3
XCM	Preoperative to 90 days	4.63	1.25	4.5	3.6	6
FGG	Preoperative to 90 days	3.64	2.01	4.3	2.1	4.7
Control	Preoperative to 90 days	0.13	0.312	0	0	0